Cloning PCR Procedures

Prep Protocol:

1. Dilute primers
    a. dilute primers to 10uM, resuspend with H2O buffer (same as the one for mini prep)
    b. stock primer concentration 100 uM
        i. trick: solvent quantity in uL - multiple number of original product nM on tube by 10
        ii. to make 10uM for PCR, add 90uL H2O to 10uL 100uM stock primer
    c. store DNA content at -20C freezer

Cloning PCR protocol:

2.5ul 10uM forward primer
2.5ul 10uM reverse primer
1ul 10mM DNTP
10ul 5X HF buffer
10ng template
0.5ul PHUSION DNA polymerase
add dH2O for total volume 50uL

Machine settings:

98C 30sec
25 cycles

- 98C 10 sec denaturing
- 58C 30sec annealing
- 72C Xsec extending
    - Phusion polymerase extends at 2kb/min
    - 750bp - 45s (2kb/min + 15sec)

72C 10 minutes
4C infinity